# Articles

# The Incidence of *Chlamydia pneumoniae* Lower Respiratory Tract Infections Among University Students in Northern California

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**_Chlamydia pneumoniae_ has recently been identified as a cause of lower respiratory tract infections. From March 1987 to March 1988, 259 university students—151 students with lower respiratory tract infections and 108 controls—from the University of California, Berkeley, were studied to determine the incidence and pattern of _C pneumoniae_ lower respiratory tract infections. Serologic evidence of a recent _C pneumoniae_ infection was found in less than 2%, and the organism was not isolated from any of the subjects. Despite the paucity of evidence of a recent infection, 47.5% of this university population showed serologic evidence of a previous _C pneumoniae_ infection. The lower incidence of _C pneumoniae_ infection in our population, when compared with previous reports, suggests that there may be geographic and temporal differences or fluctuations among populations.**

(Katzman DK, Tipton AC, Litt IF, Friedman IM, Emmons RW, Schachter J: The incidence of *Chlamydia pneumoniae* lower respiratory tract infections among university students in northern California. West J Med 1991 Aug; 155:136-139)

*Chlamydia pneumoniae*, a new species within the genus *Chlamydia*, has recently been identified as a cause of acute respiratory tract infection including pneumonia.[1-6] The organism was originally called TWAR after the laboratory designation of two early isolates, TW 183 and AR 39. TWAR strains had been tentatively classified as *Chlamydia psittaci* based on their inclusion morphology in HeLa cells and because of their failure to stain with iodine.[6-8] In a study at the University of Washington (Seattle) from 1984 through 1986, the TWAR organism was identified in 8 of 13 students with pneumonia who had serologic evidence of TWAR infection. This study was particularly important because the recovery of the TWAR organism reduced the likelihood that the serologic findings reflected polyclonal stimulation or cross-reactive antigens from other bacteria. Thus, the study helped establish the etiologic role of TWAR in acute respiratory tract disease.[1]

Seroepidemiologic studies have provided evidence that *C pneumoniae* causes human infection more often than any other chlamydia, including all *Chlamydia trachomatis* serovars. *C pneumoniae* antibody has been found in 25% to 50% of adults in eight different areas of the world.[1-6,9-12] *C pneumoniae* antibody is infrequently found in children younger than 5 years, and the prevalence increases sharply during the ages of 10 to 30 years and persists into old age.[1,13-17]

In this article we report the results of a prospective, cross-sectional, descriptive study designed to determine the incidence and pattern of *C pneumoniae* lower respiratory tract infections in a university population.

## Subjects and Methods

### *Subjects*

All subjects were students at the University of California, Berkeley, between the ages of 18 and 35 years who were seen at the Student Health Service from March 1987 to March 1988. Two groups of subjects were studied. The "symptomatic" group included those subjects who had lower respiratory tract illness defined by one of the following criteria: cough, shortness of breath, chest pain, breathing fast, or hoarseness. The "asymptomatic" group included subjects who were seen at the same general medical clinic as the symptomatic subjects and presented with a variety of acute medical complaints. The asymptomatic group served as controls because they had not had a febrile or respiratory illness within the past two weeks. Students were excluded if they had received antibiotics within the previous three weeks. The symptomatic and asymptomatic groups were enrolled simultaneously throughout the year.

Power calculations were carried out to determine the necessary sample size. With a power of 0.9 and a predicted 10% difference in prevalence of *C pneumoniae* between the symptomatic and asymptomatic groups (15% versus 5%, respectively), 200 subjects would be required in each group.

Institutional approval regarding the participation of human subjects was obtained. Informed consent was obtained before enrollment in the study. A self-administered questionnaire was completed and a physical examination was done on each symptomatic patient.

### *Specimens*

Three specimens from the posterior oropharynx were obtained from each symptomatic subject. One specimen was placed in chlamydia collection medium.[18] The second specimen was placed in viral transport medium,[19] and the third was smeared on a microscopy slide and fixed for the Ortho Chlamydia Direct Detection fluorescent-antibody collection kit. This method uses a fluorescein-conjugated monoclonal antibody to a genus-specific chlamydial antigen for detecting chlamydial elementary bodies.[20]

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This project was supported in part by Public Health Service grant No. AI21912 from the National Institutes of Health and the Robert Wood Johnson Foundation, General Pediatrics Academic Development Fellowship Program grant No. 10068.

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A single swab of the posterior oropharynx was obtained from each asymptomatic subject and placed in chlamydia collection medium.[18]

Cultures for *C trachomatis* of specimens from the cervix and male urethra were not done.

*Serology*

Serum specimens of the acute and convalescent phases were drawn four weeks apart. Attempts were made to obtain both serum specimens from all patients. The microimmunofluorescence test[21] with immunoglobulin (Ig) M and IgG conjugates[22] was used for both the acute and convalescent serum. The antigens used included elementary bodies of all 15 *C trachomatis* serovars[23] and the *C pneumoniae* strain TW183. Serologic evidence of a recent infection was defined as a fourfold rise in antibody titer or a titer of 1:32 or greater in the IgM serum fraction. Serologic evidence of a previous infection was defined as a titer that was greater than 1:16 in the IgG serum fraction.

*Isolation*

Chlamydia pneumoniae *and* Chlamydia trachomatis. All specimens were processed in a cycloheximide-treated McCoy cell system.[24] A blind passage was done four days after inoculation. Cover slips for both passages were assessed for inclusions using a genus-specific, fluorescein-conjugated monoclonal antibody. Selected specimens (those from patients with high antibody levels) were also inoculated into HeLa cells and the yolk sac of embryonated hens' eggs and processed using standard procedures for identification of chlamydiae.[18]

Mycoplasma pneumoniae *and respiratory viruses.* Isolation and complement-fixation tests for *Mycoplasma pneumoniae,* influenza viruses A and B, respiratory syncytial virus, adenovirus, Q fever, parainfluenza virus types 1, 2, and 3, and chlamydia were carried out by the Viral and Rickettsial Disease Laboratory, California Department of Health Services, using standard methods.[19]

## Results

A total of 259 subjects were recruited, 151 with lower respiratory tract illness and 108 control subjects. The proportion of all students seen at the Student Health Service with respiratory infection during this study period is unknown. About 5% refused enrollment because of time constraints or refusal of venipuncture or both. Of the 259 participants, 96.1% returned for a follow-up titer. Subjects ranged in age from 18 to 35 years, with a mean of 22.2 years (SD = 3.6). The study population was 55.2% men and 44.8% women.

Of the 151 subjects with lower respiratory tract illness, 2 (1.3%) had serologic evidence of an acute or recent *C pneumoniae* infection. The *C pneumoniae* organism was not isolated from either of these patients. There was neither serologic evidence of an acute *C pneumoniae* infection nor positive oropharyngeal cultures in the asymptomatic or control group.

The following reports describe the cases of two patients with serologic evidence of an acute *C pneumoniae* infection.

*Case 1*

The patient, a 19-year-old woman, sought care in October 1987 because of a sore throat, fever, and cough. The cough began about a month before her visit and was noted to be productive of yellow mucoid sputum. She also had hoarseness and "red eyes." The patient said she did not smoke. She lived in a campus dormitory and had no contact with birds or animals.

TABLE 1.—*Results of Tests on Acute and Convalescent Serum and Oropharyngeal Culture in 2 Patients With Lower Respiratory Tract Illness and Acute* Chlamydia pneumoniae *Antibody*

| Patient | Chlamydia pneumoniae Antibody IgM acu/con* | Chlamydia pneumoniae Antibody IgG acu/con* | Culture Results | Antibody to Other Etiologic Agents |
|---|---|---|---|---|
| 1..... | >256/64 | 128/128 | Negative | None |
| 2..... | 0/128 | 128/>256 | Negative | None |

*Acute titer versus convalescent titer.

On physical examination, her vital signs were stable and she was afebrile. The conjunctival linings of both eyelids were red and injected. There was slight tearing without mucopurulent discharge. Her pharynx was erythematous with a small amount of exudate on the right tonsil. She had an occasional expiratory wheeze on auscultation. Her leukocyte and differential counts were normal. No chest radiograph was taken. The patient was treated with a regimen of 1 gram per day of erythromycin ethylsuccinate for 10 days, returned for her repeat serum specimen 34 days after her initial visit, and was subsequently lost to follow-up.

*Case 2*

The patient, a 25-year-old woman, was seen in November 1987 because of fever, sore throat, hoarseness, and cough for six days. Her cough was productive of clear mucus. A few days before her visit to the Student Health Service, shortness of breath developed. The patient said she did not smoke cigarettes. She shared an apartment with other students, all of whom were healthy. This patient did not have pets or work with animals.

On physical examination, her vital signs were stable and she was afebrile. Her voice was hoarse. The conjunctival linings of both eyelids were red and injected but without discharge. On auscultation there were diffuse rhonchi throughout the bases of both lung fields. The findings of the examination were otherwise entirely normal. A chest radiograph and blood work were not done, and a regimen of doxycycline hyclate, 100 mg twice a day for seven days, was started. She returned for her second serum specimen 27 days after her initial visit. No follow-up was documented.

The results of both of these patients' oropharyngeal cultures and the acute and convalescent serum specimen values to *C pneumoniae* antibody are shown in Table 1. Both patients were from the symptomatic group. No other etiologic agent was identified in either case.

This university study population included 123 subjects (47.5%) with IgG antibodies to *C pneumoniae*. Of these, 74 (49.0%) were from the symptomatic group and 49 (45.4%) were from the control group. Antibody to *C pneumoniae* was slightly more common among men (49.7%) than women (44.8%) (Table 2). The *C pneumoniae* organism was not isolated from any of the subjects. Similarly, all the oropharyngeal specimens tested with the direct fluorescent antibody stain for *C pneumoniae* had negative results.

Evidence of antibody to *C trachomatis* was found in one subject, a 35-year-old woman, in the acute-phase serum. An

TABLE 2.—*Results of the Microimmunofluorescent Antibody Test With* Chlamydia pneumoniae *and* Chlamydia trachomatis *Antigens in 259 University Students*

| Antibody | No. of Patients (%) | | | |
|---|---|---|---|---|
| | Symptomatic, n = 151 | Asymptomatic, n = 108 | Men, n = 143 | Women, n = 116 |
| *C pneumoniae* | | | | |
| Acute | 2 (1.3) | 0 | 0 | 2 (1.7) |
| Chronic | 74 (49.0) | 49 (45.4) | 71 (49.7) | 52 (44.8) |
| *C trachomatis* | | | | |
| Acute | 1 (0.66) | 0 | 0 | 1 (0.9) |
| Chronic | 45 (29.0) | 30 (27.8) | 32 (23.4) | 43 (37.1) |

IgG antibody to *C trachomatis* was found in 75 (28.9%) participants, 45 of them from the symptomatic group. There were 32 (42.7%) men and 43 (57.3%) women with IgG antibody to *C trachomatis*. This organism, however, was not isolated from the oropharynx of any of the 259 subjects.

The results of the microimmunofluorescent antibody test with *C pneumoniae* and *C trachomatis* antigens are shown in Table 2.

The results of all studies for the respiratory viruses *M pneumoniae* and *C pneumoniae* in the symptomatic subjects are shown in Table 3. There were 31 (20.5%) symptomatic subjects who had an identifiable cause for their lower respiratory tract illness. No patient had evidence of infection with more than one organism. *M pneumoniae* occurred throughout the study period. The seven cases of influenza B virus occurred in February and March 1988. Because of this high incidence, we decided to run the complement fixation serologic test for influenza B virus on the specimens of all control subjects who enrolled in the study during this time. There was no serologic evidence of recent infection due to influenza B virus in the control group.

TABLE 3.—*Laboratory Results of 151 Subjects With Lower Respiratory Tract Illness*

| Etiologic Agent | No. of Subjects, n = 151 | Diagnostic Tests* |
|---|---|---|
| *Mycoplasma pneumoniae* | 11 | Serology |
| Influenza B | 3 | Serology and Culture |
| | 2 | Culture |
| | 2 | Serology |
| Parainfluenza type 2 | 2 | Culture |
| Respiratory syncytial virus | 2 | Serology |
| Parainfluenza type 1 | 1 | Culture |
| Influenza A | 1 | Serology |
| Adenovirus | 1 | Serology |
| Herpesvirus simplex type 1 | 1 | Culture |
| Rhinovirus | | |
| type 13 | 1 | Culture |
| type 41 | 1 | Culture |
| type 71 | 1 | Culture |
| *Chlamydia pneumoniae* | 2 | Serology |
| Total | 31 | |

*All serologic tests done by complement fixation.

## Discussion

Lower respiratory tract disease caused by *Chlamydia pneumoniae* has been shown to occur in endemic or sporadic patterns, and reports on military and civilian populations have suggested that *C pneumoniae* may have both a low background rate of endemic infection and a cyclic epidemic pattern.[1-6,9,10] Kleemola and colleagues reported on four epidemics of pneumonia in military trainees in Finland from 1957 to 1985.[4] The pneumonia attack rate was similar in all four epidemics, ranging from 60 to 84 per 1,000 men. Each epidemic lasted five to seven months, and they occurred during all seasons of the year.[4] During civilian epidemics, 49% to 71% of patients with pneumonia having positive complement fixation tests and thought to have psittacosis actually showed evidence of current *C pneumoniae* infection, compared with only 10% to 20% in nonepidemic years.[6] It is unwise to assume that this is the case in all populations and geographic areas, however. In a study of Seattle college students, approximately 20% of patients with pneumonia had evidence of *C pneumoniae* infection.[1]

Based on this and the Scandinavian reports,[6] this organism would be expected to be a common cause of pneumonia. This certainly was not the case in our study. Only two subjects with lower respiratory tract illness had serologic evidence of a recent *C pneumoniae* infection. In neither did the organism grow in culture. We did find a high seroprevalence of antibody to *C pneumoniae* despite the paucity of evidence of recent infection. This raises the possibility of a cyclic pattern of infectivity for this organism. One of the limitations of this study is that we observed this population only over a year. Evidence for the cyclic nature of *C pneumoniae* infection would probably be much stronger had we been able to continue this study over several years.

Studies of the antibody response in isolation-proven *C pneumoniae* infection have shown that the timing of serum specimens is critical to the serologic diagnosis of *C pneumoniae* infection. Two patterns of antibody response to the *C pneumoniae* organism have been reported. That seen in younger patients consists of a diagnostic titer in the IgM serum fraction that appears after three weeks from the start of symptoms. The IgG response in this group usually does not appear before six weeks from the occurrence of symptoms. The second pattern of antibody response is seen in older persons and is presumed to be associated with secondary infection or reinfection with *C pneumoniae*. An antibody response to *C pneumoniae* in the IgG fraction appears much sooner. If the IgM fraction is positive, it tends to be at a low titer (32 or 64).[3] We attempted to obtain all our follow-up titers at four weeks from the initial visit. The time span between the acute and convalescent titers in this study ranged between 20 and 103 days. The mode and median number of days were 28 and 29, respectively. Some change in IgG antibody may have been missed because patients had their convalescent serum specimen drawn earlier than six weeks.

Previous reports have concluded that respiratory infection with *C pneumoniae* in ambulatory adolescents and young adults is a poorly recognized clinical disorder,[3,5,25,26] probably because of its mild and nonspecific clinical presentation. The infection may be completely asymptomatic.[25] The clinical features of the two persons with serologic evidence of acute *C pneumoniae* infection in this study were those of a mild lower respiratory tract infection. In the study by Grayston and associates, pneumonia associated with *C pneumoniae* was described as being clinically similar to that caused by *Mycoplasma pneumoniae*.[1] In fact, infection with *C pneumoniae* has been described as an atypical pneumonia perhaps mistakenly treated as *M pneumoniae*.[1] In the Scandinavian epidemic of 1981 to 1983, it appeared that those with

TWAR antibody titers suggestive of current infection had diagnoses similar to those without the antibody.[6] During the Finnish pneumonia epidemic caused by *C pneumoniae*, the conscripts had relatively mild illnesses and none were reported to be life-threatening.[4] It now seems possible that in these studies, much of the lower respiratory tract illness ascribed to other respiratory pathogens was actually due to *C pneumoniae*. Furthermore, it is likely that subclinical infections are caused by this organism, as are other chlamydial infections.[27] The asymptomatic group in our study, however, had no evidence of acute *C pneumoniae* infection.

*C pneumoniae* is a difficult organism to culture. Meticulous care with suitable equipment was taken during all stages of obtaining and transporting specimens. Recent reports have suggested that nasopharyngeal specimens and identification of *C pneumoniae* is a better technique than taking oropharyngeal specimens, as used in this investigation.[28] Furthermore, HL cells have been shown to be an excellent cell culture system for laboratory propagation of *C pneumoniae* when compared with other cell lines (such as McCoy, HeLa-229, or BHK-21) and may also be a more sensitive cell line for the initial identification.[29]

In our study population, *C pneumoniae* antibody was more common than antibody to *Chlamydia trachomatis*. Furthermore, the *C pneumoniae* antibody was slightly more common in men than in women (Table 2). Antibody to *C trachomatis*, on the other hand, was more common in women. These findings have been reported in other populations.[1,3,13]

*C trachomatis* is known to be a respiratory tract pathogen in children younger than 6 months and in immunocompromised hosts.[30,31] Earlier serologic studies found an association of community-acquired pneumonia and pharyngitis with *C trachomatis*, but there were no attempts to isolate *C trachomatis* from the oropharynx in any of the subjects in these studies.[32,33] It appears that some of the serologic results indicating *C trachomatis* involvement in pneumonia or pharyngitis might in fact reflect *C pneumoniae* antibody. We have found that many (but not all) of the seropositive cases represent cross-reactivity with *C pneumoniae* antigens and probably represent infection with *C pneumoniae*, rather than with *C trachomatis*.[34] At the time of the study by Komaroff and coworkers, *C pneumoniae* had not been identified. Our study, however, found no evidence that *C trachomatis* causes lower respiratory tract infections. *C trachomatis* was not identified in the oropharyngeal specimens from any of the subjects. Although studies have found that *C trachomatis* can cause 3.5% to 6.3% of pharyngitis cases in the adult population,[35,36] *C pneumoniae* is probably a more common cause of pharyngitis. Only 1 of our 259 subjects had serologic results suggestive of a recent *C trachomatis* infection. This 35-year-old woman was contacted and subsequently referred to her physician for follow-up and treatment.

There is still much to learn about the *C pneumoniae* organism. The epidemiology and annual changes in occurrence of *C pneumoniae* are still poorly understood. Further studies and better diagnostic techniques will help determine the clinical spectrum and the epidemiology of this infection.

## REFERENCES


1. Grayston JT, Kuo CC, Wang SP, Altman J: A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. N Engl J Med 1986; 315:161-168

2. Grayston JT, Kuo CC, Wang SP, et al: Clinical findings in TWAR respiratory tract infections, *In* Oriel JD, Ridgway G, Schachter J, et al (Eds): Chlamydial Infections. Cambridge, England, Cambridge University Press, 1986, pp 337-340

3. Marrie TJ, Grayston JT, Wang SP, Kuo CC: Pneumonia associated with the TWAR strain of Chlamydia. Ann Intern Med 1987; 106:507-511

4. Kleemola M, Saikku P, Visakorpi R: Epidemics of pneumonia caused by TWAR, a new *Chlamydia* organism, in military trainees in Finland. J Infect Dis 1988; 157:230-236

5. Saikku P, Wang SP, Kleemola M, Brander E, Rusanen E, Grayston JT: An epidemic of mild pneumonia due to an unusual strain of *Chlamydia psittaci*. J Infect Dis 1985; 151:832-839

6. Grayston JT, Mordhorst C, Bruu AL, Vene S, Wang SP: Countrywide epidemics of *Chlamydia pneumoniae*, strain TWAR, in Scandinavia, 1981-1983. J Infect Dis 1989; 159:1111-1114

7. Gordon FB, Quan AL: Occurrence of glycogen in inclusions of the psittacosis-lymphogranuloma venereum-trachoma agents. J Infect Dis 1965; 115:186-196

8. Moulder JW, Hatch TP, Kuo CC, et al: Chlamydia, Jones, Rake and Stearns 1945, *In* Krieg NR, et al (Ed): Bergey's Manual of Systematic Bacteriology. Baltimore, Md, Williams & Wilkins, 1984, pp 729-735

9. Grayston JT, Wang SP, Foy HM, et al: Seroepidemiology of *Chlamydia trachomatis* infection, *In* Mardh PA, et al (Eds): Chlamydia Infections: Proceedings, 5th International Symposium, Lund, Sweden, June 1982. New York, NY, Elsevier North-Holland Biomedical Press, 1982, pp 405-419

10. Mordhorst CH, Wang SP, Grayston JT: Epidemic 'ornithosis' and TWAR infection, Denmark 1976-85, *In* Oriel JD, Ridgway G, Schachter J, et al (Eds): Chlamydial Infections. Cambridge, England, Cambridge University Press, 1986, pp 325-328

11. Wang SP, Grayston JT: Microimmunofluorescence serological studies with the TWAR organism, *In* Oriel JD, Ridgway G, Schachter J, et al (Eds): Chlamydial Infections. Cambridge, England, Cambridge University Press, 1986, pp 329-332

12. Forsey T, Darougar S, Treharne JD: Prevalence in human beings of antibodies to Chlamydia IOL-207, an atypical strain of chlamydia. J Infect 1986; 12:145-152

13. Kuo CC, Chen HH, Wang SP, Grayston JT: Identification of a new group of *Chlamydia psittaci* strains called TWAR. J Clin Microbiol 1986; 24:1034-1037

14. Burney P, Forsey T, Darougar S, Sittampalam Y, Booth P, Chamberlain R: The epidemiology of chlamydial infections in childhood: A serological investigation. Int J Epidemiol 1984; 13:491-495

15. Black SB, Grossman M, Cles L, Schachter J: Serologic evidence of chlamydial infection in children. J Pediatr 1981; 98:65-67

16. Harrison HR, Phil D, Magder LS, et al: Acute *Chlamydia trachomatis* respiratory infection in childhood. Am J Dis Child 1986; 140:1068-1071

17. San Joaquin VH, Rettig PJ, Newton JY, Marks MI: Prevalence of chlamydial antibodies in children. Am J Dis Child 1982; 136:425-427

18. Schachter J: Chlamydiae (psittacosis-lymphogranuloma venereum-trachoma group), *In* Lennette EH (Ed): Manual of Clinical Microbiology, 4th Edition. Washington, DC, American Society for Microbiology, 1985, pp 856-862

19. Lennette EH, Schmidt NJ (Eds): Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections. Washington, DC: American Public Health Association, 1979, pp 35-42

20. Bell TA, Kuo CC, Stamm WE, et al: Direct fluorescent monoclonal antibody stain for rapid detection of infant *Chlamydia trachomatis* infections. Pediatrics 1984; 74:224-228

21. Wang S, Grayston JT, Alexander ER, et al: Simplified microimmunofluorescence test with trachoma-lymphogranuloma venereum *(Chlamydia trachomatis)* antigens for use as a screening test for antibody. J Clin Microbiol 1975; 1:250-255

22. Wang SP, Grayston JT, Kuo CC, et al: Serodiagnosis of *Chlamydia trachomatis* infection with the micro-immunofluorescence test, *In* Hobsen D, Holmes KK (Eds): Nongonococcal Urethritis and Related Infections. Washington, DC, American Society for Microbiology, 1977, pp 237-248

23. Wang SP, Kuo CC, Grayston JT: Formalized *Chlamydia trachomatis* organisms as antigen in the micro-immunofluorescence test. J Clin Microbiol 1979; 10: 259-261

24. Ripa KT, Mardh PA: Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. J Clin Microbiol 1977; 6:328-331

25. Saikku P, Visakorpi R, Kleemola M, et al: *Chlamydia psittaci* TWAR-strains as a frequent cause of military pneumonia epidemics in Finland, *In* Oriel JD, Ridgway G, Schachter J, et al (Eds): Chlamydial Infections. Cambridge, England, Cambridge University Press, 1986, pp 333-336

26. Schachter J: Human *Chlamydia psittaci* infection, *In* Oriel JD, Ridgway G, Schachter J, et al (Eds): Chlamydial Infections. Cambridge, England, Cambridge University Press, 1986, pp 311-320

27. Grayston JT: *Chlamydia pneumoniae*, strain TWAR. Chest 1989; 95:664

28. Berdal BP, Fields PI, Mitchell SH, et al: Isolation of *Chlamydia pneumoniae* during an adenovirus outbreak, *In* Bowie WR, Caldwell HD, Jones RP, et al (Eds): Chlamydial Infections—Proceedings of the Seventh International Symposium on Human Chlamydial Infections. Cambridge, England, Cambridge University Press, 1990, pp 445-448

29. Cles LD, Stamm WE: Use of HL cells for improved isolation and passage of *Chlamydia pneumoniae*. J Clin Microbiol 1990; 28:938-940

30. Beem MO, Saxon EM: Respiratory-tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. N Engl J Med 1977; 296:306-310

31. Tack KJ, Peterson PK, Rasp FL, et al: Isolation of *Chlamydia trachomatis* from the lower respiratory tract of adults. Lancet 1980; 1:116-120

32. Komaroff AL, Aronson MD, Schachter J: *Chlamydia trachomatis* infection in adults with community-acquired pneumonia. JAMA 1981; 245:1319-1322

33. Komaroff AL, Aronson MD, Pass TM, Ervin CT, Branch WT Jr, Schachter J: Serologic evidence of chlamydial and mycoplasmal pharyngitis in adults. Science 1983; 222:927-929

34. Komaroff AL, Branch WT Jr, Aronson MD, Schachter J: Chlamydial pharyngitis (Letter). Ann Intern Med 1989; 111:537-538

35. Jones RB, Rabinovitch RA, Katz BP, et al: *Chlamydia trachomatis* in the pharynx and rectum of heterosexual patients at risk for genital infection. Ann Intern Med 1985; 102:757-762

36. McDonald CJ, Tierney WM, Hui SL, French ML, Leland DS, Jones RB: A controlled trial of erythromycin in adults with nonstreptococcal pharyngitis. J Infect Dis 1985; 152:1093-1094